A Screening Method for Liver Glycogen Diseases

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The majority of patients with glycogenosis of the liver have one of three types of enzyme deficiency: deficiency of glucose-6-phosphatase, of the debranching enzyme system, or of the phosphorylase system (Illingworth, 1961; Hers and Van Hoof, 1968). The severity of the disease and of its manifestations, such as hypoglycaemia and acidosis, is related to the underlying enzyme defect (van Creveld and Huijing, 1965).

Treatment of patients with liver glycogenosis is dietary. The diet aims at reducing the effects of the metabolic disturbance and must vary with the type of enzyme defect (Fernandes and van de Kamer, 1965, 1968). It is important to start dietary management as early as possible, and therefore to diagnose the disease and the enzyme deficiency involved, at an early age. The definitive diagnostic procedure is assay of the enzymes, either in liver tissue or—for debranching enzyme and phosphorylase—in the leucocytes. But it would be useful to have available simpler tests to screen children in whom liver glycogen disease is suspected.

Various screening procedures have been recommended, such as the epinephrine tolerance test (Howell, Ashton, and Wyngaarden, 1962), glucagon tolerance test (Hug, 1962; Lowe *et al.*, 1962), and intravenous tolerance test with fructose (Hers, 1959) or galactose (Schwartz, Ashmore, and Renold, 1957). Öckerman (1967) has reviewed the application of these tests. In our experience, they often have drawbacks. With the glucagon test, all our patients with deficiency of the phosphorylase system showed a rise in blood glucose no different from that of normal children. Thus, the statement of Sokal, Lowe, and Sarcione (1962) that a rapid rise of 50 mg./100 ml. or more in blood glucose after intramuscular glucagon virtually rules out liver glycogen disease, does not hold for this group of patients. Moreover, the glucagon test is usually performed after prolonged fasting

and is thus potentially dangerous in conditions where glucagon fails to cause glucose release by the liver. With glucose-6-phosphatase deficiency the increased production of lactic acid entails the additional risk of acidosis. Fructose and galactose tolerance tests involve serious risk in the case of glucose-6-phosphatase deficiency, because these patients convert the sugars not into glucose but into lactic acid (Fernandes and van de Kamer, 1965).

Children with glucose-6-phosphatase deficiency have high fasting lactate levels which fall after glucose administration (Howell *et al.*, 1962; Lowe *et al.*, 1962). This fact, together with our earlier observations (Fernandes and van de Kamer, 1965, 1968), that children with deficiency of the debranching enzyme or of the phosphorylase systems respond to carbohydrate administration with an abnormal rise of blood lactate, suggested that the blood lactate concentration could be used for diagnostic screening. We have therefore investigated the lactate response during oral hexose tolerance tests in patients with the three types of liver glycogen disease mentioned. These lactate curves, together with the response of blood glucose to glucagon, enable the underlying type of glycogen disease to be identified.

Patients and Methods

The studies were carried out in 5 children with glucose-6-phosphatase deficiency, 5 children with a deficiency of the debranching enzyme system, and 8 children with a deficiency of the phosphorylase system. In the case of glucose-6-phosphatase deficiency the diagnosis was confirmed by enzymic assay of liver tissue (Hers, 1964), in the latter two groups by enzymic assay of the leucocytes (Huijing, 1964a, b, 1967; Huijing, Klein Obbink, and van Creveld, 1968). The relevant clinical and biochemical data on the 18 patients are presented in the Table.

Patients with a deficiency of the phosphorylase system fall into two groups; those with a low phosphorylase kinase activity in leucocytes and erythrocytes, and those

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TABLE
Enzyme Assays in 18 Children with Liver Glycogen Disease*

Case No.	Sex, age (yr.)	Glucose-6-phosphatase in Liver (μ moles phosphate produced from glucose-6-phosphate/min. per g. liver)	Debranching Enzyme in Leucocytes (n moles glucose produced from phosphorylase limit dextrin/min. per mg. protein)	Phosphorylase in Leucocytes (n moles glucose-1-phosphate produced/min. per mg. protein)		Phosphorylase b Kinase in Leucocytes (U phosphorylase b activated/min. per mg. protein)	Remarks
				AMP -	AMP +		
1	F, 14	0		22			Gout
2	F, 4	0					Sister of Case 2
3	F, 2	0	0.16	24	26		
4	M, 4	0.08	0.45	15	35		
5	F, 1	0.08	0.14	19	17		
6	F, 8		0.0	24	26		Sister of Case 6
7	F, 7		0.0	18	21		
8	F, 3		0.0	18	23		
9	F, 5	1.5	0.0	17	19		
10	F, 1		0.0	30	29		
11	M, 6	4.8	0.47	5	15	0.57	Brother of Case 7
12	M, 8			5	33	0.06	
13	F, 3	0.8	0.32	2	17	0.16	
14	M, 2		0.31	5	21	0.02	
15	M, 4		0.39	3	22	0.12	
16	M, 1		0.21	3	21	0.08	
17	M, 4		0.62	1	23	0.06	
18	M, 1		0.40	3	20	0.00	
Normal		2-10	0.12-0.70	12-50	15-50	0.30-1.2	

* Italicized figures indicate the enzyme deficiency involved.

with normal phosphorylase kinase activity. In both groups it is only in the absence of adenosine 5'-monophosphate (AMP) that there is consistently low phosphorylase activity in leucocytes. All patients with a deficiency of the phosphorylase system, except Case 11, had a low phosphorylase kinase activity.

The patients with glucose-6-phosphatase deficiency were given an oral glucose tolerance test and a glucagon test; fructose and galactose tolerance tests were not performed because of the undue risk involved. The patients with debranching enzyme deficiency and phosphorylase deficiency completed oral tolerance tests with glucose, fructose, and galactose, and a glucagon test. The sugars were administered orally as a 10% solution in a dose of 2 g./kg. with a maximum of 50 g. The fasting period before the test was at least 8 hours, except in 2 children with glucose-6-phosphatase deficiency (Cases 4 and 5) whose low preprandial blood glucose levels did not permit so long a fast. Glucagon was administered intramuscularly in a dose of 0.5-1 mg. depending on the age of the child. During the tests 0.4 ml. samples of capillary blood were taken at regular intervals for the determination of lactate and glucose. Blood lactate was estimated with lactate dehydrogenase, blood glucose with glucose oxidase, using the reagent sets TC-B and TC-M (Boehringer, Mannheim, Germany).

Results

Glucose tolerance tests. Fig. 1 shows lactate levels during a glucose tolerance test carried out in

the 5 patients with glucose-6-phosphatase deficiency, and a control curve. Fasting lactate levels varied greatly among the patients—the preceding fasting period also varied—but in all the levels were raised. After glucose administration the lactate level decreased steadily, in one patient after a lag of 1 hour. In this case the test was performed 4 hours after the last meal. The decrease of the lactate levels in the patients contrasts with the slight increase, from a low initial level, in the controls.

Fig. 2 shows the lactate levels during a glucose tolerance test carried out in the 5 patients with debranching enzyme deficiency and the 8 patients with phosphorylase deficiency. The fasting lactate levels of the patients were in the normal range. After glucose challenge the lactate levels in most of the patients increased significantly. This was always so in the children with debranching enzyme deficiency, whose curves never fell within the normal range. In patients with a phosphorylase deficiency the lactate curves were more divergent, and in 2 patients they remained within the normal range.

Fructose tolerance tests. Fig. 3 shows lactate levels during a fructose tolerance test carried out in the patients with debranching enzyme deficiency and phosphorylase deficiency,

FIG. 1.—Blood lactate concentrations during oral glucose tolerance tests in 5 children with glucose-6-phosphatase deficiency. The control curve is the average $\pm$ 2 SD from 12 normal children.

and a control curve. Again the lactate curves of most patients showed a marked increase, though they were divergent. There was no overlapping of the curves of patients and normals, but one curve of a patient with phosphorylase deficiency lay near the upper limit of the normal range.

Galactose tolerance tests. Fig. 4 shows lactate levels during a galactose tolerance test carried out in the patients with debranching enzyme deficiency and phosphorylase deficiency, and a control curve. The lactate curves of all patients showed a marked increase, and there was no overlapping of the curves of patients and normals.

Glucagon tolerance tests. Fig. 5 shows glucose levels during a glucagon tolerance test performed on the patients and 7 normal children. Patients with phosphorylase deficiency responded to glucagon with a distinct rise in glucose level. The glucose curves of these children could not be distinguished from the normal. In patients with glucose-6-phosphatase deficiency and with debranching enzyme deficiency, however, glucagon had little or no effect on the blood glucose levels, and in 4 children with glucose-6-phosphatase deficiency the glucose levels were even exceptionally low.

Discussion

It is clear that an abnormal blood lactate response to hexose challenge is characteristic of most

FIG. 2.—Blood lactate concentrations during oral glucose tolerance tests in (a) 5 children with deficiency of the debranching enzyme system and (b) 8 children with deficiency of the phosphorylase system. Same control curve as in Fig. 1.

FIG. 3.—Blood lactate concentrations during oral fructose tolerance tests in (a) 5 children with deficiency of the debranching enzyme system and (b) 8 children with deficiency of the phosphorylase system. The control curve is the average $\pm$ 2 SD from 11 normal children.

patients with liver glycogen disease. The high fasting lactate levels in children with glucose-6-phosphatase deficiency can be readily explained, for here the degradation of glycogen fails to result

in the liberation of glucose, increased breakdown of accumulated glucose-6-phosphate into pyruvate and lactate taking place instead. Lactate production is stepped up as glucose hunger increases,

FIG. 4.—Blood lactate concentrations during oral galactose tolerance tests in (a) 5 children with deficiency of the debranching enzyme system and (b) 8 children with deficiency of the phosphorylase system. The control curve is the average $\pm$ 2 SD from 9 normal children.

FIG. 5.—Blood glucose concentrations after intramuscular glucagon in (a) 5 children with glucose-6-phosphatase deficiency, (b) 5 children with deficiency of the debranching enzyme system, (c) 7 children with deficiency of the phosphorylase system and (d) 7 normal children.

while glucose administration halts the increased glycogenolysis, and the lactate level accordingly falls.

The lactate increase following hexose feeding to children with debranching enzyme or phosphorylase deficiency is more difficult to understand. We suppose that it must be considered as an overflow phenomenon because of increased glycolysis, just as ketosis is an overflow because of increased lipolysis. In addition, decreased glycogen synthesis may play a role. This hypothesis is supported by the following facts. (1) Glycogen synthetase activity in the leucocytes of these patients is sub-normal (Huijing, 1964a), possibly as a result of hormonal effects. This may also be the case in the liver, where the very high glycogen concentration may play an additional regulatory role. Thus,

glycogen deposition from exogenous carbohydrate would be limited and as a consequence glycolysis would be enhanced. (2) More lactate is produced after galactose or fructose challenge than during the glucose test. Galactose and fructose are almost exclusively metabolized in the liver, where they are transformed into glucose or broken down to pyruvate and lactate, whereas glucose can bypass the liver and be metabolized directly by the peripheral tissues. Therefore, if the patient has been loaded with glucose, hepatic glycolysis with overflow of lactate should be less than with any of the other hexoses. Moreover, we have evidence (unpublished) that in these patients there is a daily rhythm: during the day the lactate level and the lipaemia induced by carbohydrate increase gradually, to decrease again during the night when the deficient glycogenolysis is partly compensated by extensive gluconeogenesis from proteins.

Screening procedure. The results shown in Fig. 1–5 indicate that oral hexose tolerance tests with blood lactate estimations, in combination with a glucagon tolerance test, may be used to screen children with hepatomegaly for the three main types of liver glycogenosis. We recommend the diagnostic procedure indicated in Fig. 6. First, the patient is subjected to a glucose tolerance test. The outcome will be one of three types of lactate curves.

(a) The fasting lactate level is high and decreases steadily after the challenge (Fig. 6, upper middle).

In this case the diagnosis is glucose-6-phosphatase deficiency. No further tests are needed.

(b) The fasting lactate level is normal and increases markedly after the challenge (Fig. 6, upper left). This indicates either a debranching enzyme deficiency or a phosphorylase deficiency. A glucagon tolerance test is required additionally (Fig. 6, lower left). The outcome may be either: (i) a flat glucose curve indicative of debranching enzyme deficiency, or (ii) a normal glucose curve indicative of phosphorylase deficiency.

(c) The lactate curve is in the upper normal range (Fig. 6, upper right). The child either has no liver glycogen disease or a phosphorylase deficiency. An additional test is needed, preferably a galactose tolerance test (Fig. 6, lower right). A lactate increase which is significantly above normal indicates phosphorylase deficiency.

A screening procedure like ours can only yield a tentative diagnosis which needs confirmation by an enzyme assay in leucocytes, but it allows a selection of the patients in whom an enzyme assay should be carried out. It also allows early

FIG. 6.—Screening procedure for differentiating patients (P) with deficiency of glucose-6-phosphatase, deficiency of the debranching enzyme system, deficiency of the phosphorylase system, and normal children (N).

detection of the disease and a differentiation of the three main types of enzyme defect. In puzzling cases of hepatomegaly one of the rarer types of enzyme deficiency should be sought. The only other known hepatic disease characterized by raised blood lactate levels is infantile lactic acidosis (Hartmann *et al.*, 1962), which can easily be distinguished from liver glycogenosis.

Summary

Blood lactate levels were followed during oral hexose tolerance tests in 18 patients with different types of liver glycogenosis. 5 patients with glucose-6-phosphatase deficiency were given a glucose tolerance test, 5 patients with a deficiency of the debranching enzyme system, and 8 patients with a deficiency of the phosphorylase system were given glucose, fructose, and galactose tolerance tests. All patients were also given a glucagon tolerance test, with estimation of blood glucose levels. Using these tests a screening procedure for the three commonest types of liver glycogen disease has been developed.

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